1860250-2/23

GenCatch™ Viral RNA Miniprep Kit

User's Guide for

Total RNA Extraction From Virus

For Research Use Only

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Quick Start Procedure

For Experienced Users Only.

The following flow chart is only good as a quick reminder for key steps and buffers to be used. Procedure varies depending on different type of samples. First time users are strongly recommended to read through the detailed instruction protocol in section 4.

Before you start:

WARNING: strong acids and oxidants (bleach, for example) should not be used together with RXV buffer (because this kind of reaction would produce cyanide)!

Add 60 ml (for Cat. No. 1860050, 50 preps) or 180 ml (for Cat. No. 1860250, 250 preps) 98-100% ethanol to Buffer WS before use (see instructions on bottle label).

	150 µl sample
Sample Lysis:	**570 µl RXV (Carrier Added) 10 min. @RT**
Prepare for Binding:	**570 µl EtOH**
Bind to Column:	**Load and Spin in RNA Column**
Wash with:	**500 µl WS**
Wash with:	**500 µl WS**
Remove residual EtOH:	**Centrifuge 3'**
Elute DNA in:	**30-50 µl H2O**
	Down Stream Application

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Overview

GenCatch™ Viral RNA Extraction System enables efficient viral RNA extraction from non-segmented RNA virus, such as Covid-19, HIV, HCV (not for Influenza, Adenovirus and other segment RNA genome virus). Samples of various forms ranging from biological fluids, serum, plasma, body fluids, and cell culture supernatant can be used. The system utilizes silica membrane spin column technology and requires no phenol/chloroform extraction. Viral RNA purified has a A260/A280 ratio between ~1.9~2.1.

Binding Capacity:
The binding capacity of the GenCatch™ viral RNA mini column is 100 μg.

Downstream Applications:

- Northern-blotting
- RT-PCR/qPCR
- PolyA+ RNA selection
- cDNA synthesis
- Primer Extension
- In vitro translation
- Array analysis
- NGS

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Product Contents

GenCatch™ Viral RNA Extraction Miniprep Kit contains sufficient reagents for 50 (Cat. No. 1860050) and 250 (Cat. No. 1860250) viral RNA extraction applications, respectively.

Catalog Number	1860050	1860250
RXV Buffer	35 ml	190 ml
WS Buffer	15 ml	45 mlx2
RNA Carrier	1	1
Proteinase K concentrated	10 mg	3x 10 mg
RNase-free ddH2O	6 ml	27 ml
GenCatch™ Column	50 pieces	250 pieces
Collection Tube	50 pieces	250 pieces

WARNING, strong acids and oxidants (bleach, for example) should not be used together with RXV buffer (because this kind of reaction would produce cyanide)!

Add 60 ml (for Cat. No. 1860050, 50 preps) or 180 ml (for Cat. No. 1860250, 250 preps) 98-100% ethanol to Buffer WS before use (see instructions on bottle label).

If precipitation forms by freezing temperature on any buffer, warm up at 37°C to redissolve.

Storage Conditions:
Store at room temperature

All components are guaranteed for 12 months from the date of purchase, when stored under specified conditions and used as described in this manual. Long term storage of some of the buffer may harden the HDPE plastic bottle. However this will not adversely affect the performance of the kit. GenCatch™ columns should be kept sealed in the zip lock bag provided during storage and away from any heating source.

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Protocol

First time users are strongly recommended to read through this detailed protocol instruction.
For technical support please reach us at support@epochlifescience.com

Before you start:
Add 60 ml (for Cat. No. 1860050, 50 preps) or 180 ml (for Cat. No. 1860250, 250 preps) 98-100% ethanol to Buffer WS before use (see instructions on bottle label).

If precipitation forms by freezing temperature on any buffer, warm up at 37°C to redissolve.

I. Protocol:

Note: Preheat RNase-free ddH2O to 80°C.

1. Add RNA carrier to RXV Buffer.
   Add 1 ml RXV Buffer to the RNA carrier tube, vortex to dissolve and transfer to the RXV Buffer bottle, store at 4°C.

2. Pipet 150 µl sample (serum, plasma, body fluids, and cell culture supernatant) into a 1.5 ml tube.

3. Add 570 µl of carrier added RXV Buffer to the sample, mix by vortexing.
   Through mixing is required for sample lysis. If the sample volume is larger than 150 µl, increase the amount of RXV Buffer proportionally.

4. Incubate the vortexed sample at room temperature for 10 minutes.

5. Add 570 µl of ethanol (96-100%) to the sample, and mix by vortexing.
   If the starting sample is larger than 150 µl, increase the amount of ethanol proportionally.

6. Place a Total RNA Column in a 2 ml Collection Tube, apply 650 µl of the ethanol added sample from step 5 to the Total

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RNA Column, close the cap, centrifuge at 6,000 x g (8,000 rpm) for 1 minute, and discard the filtrate.
If the solution remains above the membrane, centrifuge again at 13,000 rpm.

7. Repeat step 6 for the rest of the sample.

8. Wash the column twice with 500 µl of ethanol added WS Buffer by centrifuging at full speed (13,000 rpm or 10,000 x g) for 1 minute, and discard the filtrate.
Add 60 ml (for Cat. No. 1860050, 50 preps) or 180 ml (for Cat. No. 1860250, 250 preps) 98-100% ethanol to Buffer WS before use (see instructions on bottle label).

9. Centrifuge at full speed for 3 minutes to remove traces of WS Buffer.
Residual ethanol may inhibit reverse transcriptase activity.

10. Transfer the column to a RNase-free 1.5 ml Elution Tube, add 50 µl of preheated (80°C) RNase-free ddH2O, and centrifuge at full speed for 1 minute to elute RNA.

11. Store RNA at -70°C.

II. Removal of genomic DNA in eluted total RNA by DNase:

1. Incubate total RNA with RNase-free DNase I (1 unit per µg of total RNA) in 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, and 50 µg/ml BSA at 37 °C for 15-30 minutes.

2. Remove DNase I by adding an equal volume of phenol: chloroform (1:1) and mix well. Centrifuge for 5 minutes. Transfer the upper aqueous layer to a new eppendorf tube.

3. Add 1/10 volume of 3 M sodium acetate (pH 5.2) and 1 volume of ice-cold isopropanol to the solution and mix well. Chill on ice for 30 minutes.

4. Centrifuge for 10 minutes at 4°C. Discard the supernatant. Wash the pellet twice with 1 ml of 70 % ethanol and centrifuge again.

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5. Remove all supernatant. Air dry the RNA pellet. Redissolve RNA in RNase-free ddH_2O.

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5 Trouble Shooting

The following guide addresses some of the most common problems. For more specific questions please reach us at support@epochlifescience.com

Little or no RNA eluted:

a. Insufficient sample lysis:
   Throughly mix the sample.
   Increase the incubation time to 30 min.
   Increase the incubation temperature.

b. Clogged column:
   Reduce the amount of starting sample and improve sample lysis as sugusted in a.

c. RNA is degraded:
   Use fresh samples or samples that have been stored properly. Avoid RNase contamination.

d. RNase contamination:
   Use RNase-free solution, pipette tips and centrifuge tubes.

DNA contamination:

Refer to Protocol section II: "Removal of genomic DNA in eluted total RNA by DNase"

A260/A280 ratio of eluted RNA is low:

a. Use ddH_2O of acidic pH to dilute RNA sample for spectrophotometric analysis:
   Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute RNA sample.

b. DNA is copurified with RNA:
   Refer to protocol section II: "Removal of genomic DNA in eluted total RNA by DNase"

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